Molecular Mechanics Study of Transannular Amine–Ketone (N → C═O) Interaction in Medium-Sized Heterocycles

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ABSTRACT: Medium-sized nitrogen-containing heterocycles have considerable potential as structurally novel templates for new medicinal agents. In order to evaluate this potential and to investigate their binding to various target receptors, satisfactory modeling of the properties of such compounds with force-field based computational methods is required, especially the conformations accessible to the molecules at and around their global minimum conformation. This is currently only possible with selected force fields for compounds that show a special intramolecular interaction such as the transannular interaction between a basic nitrogen atom and a carbonyl carbon atom. This article substantiates this claim and discusses two approaches to modify the commercially available CFF91 force field. The different approaches are discussed and assessed by their performance in reproducing the conformation in the crystal for a series of known model compounds. In summary, very good agreement with the experimental structure is achieved. The modified force fields are then used to investigate a potentially bioactive lead compound. The lead compound is predicted to be able to mimic the shape of a fused-ring compound with biological activity. © 1997 by John Wiley & Sons, Inc.
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Introduction

Like many other receptors, adrenergic receptors are known to have a range of pharmacologically characterized subtypes.[1,2] However, there are very few subtype selective drugs currently available. For example, α_{1A}-adrenoceptors in the human prostate are implicated in the bladder outlet obstruction seen in benign prostatic hyperplasia. Selective antagonists to this receptor subtype could lead to drugs for the treatment of this disease[3] that would show fewer side effects than currently used nonselective drugs such as prazosin.

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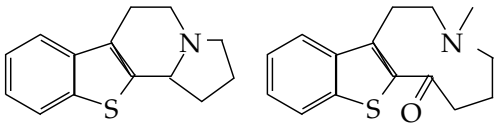
Selective α_2 antagonists are also of clinical interest as antidepressants.[4] The design principles involved in the achievement of such fine tuning of ligand–receptor affinity are far from clear. We recently published a study on the development of pharmacophoric features to distinguish antagonists showing some selectivity for either the α_{1A} or the α_{1B} adrenergic receptors.[5] The situation is similar in the subtypes of the α_2 adrenergic receptor with many of the most active ligands, such as prazosin and WB4101, binding to at least some subtypes of both α_1 and α_2 receptors.[3] Furthermore, there is considerable overlap between adrenergic activity and activity at other bioamine receptors, such as dopaminergic receptors. A comprehensive review on α- and β-adrenoceptors was recently published.[6,7]

To design selective ligands structural information on the receptor subtypes, especially their binding sites, is invaluable. There is, however, no X-ray structure available for any bioamine receptor.

One way of exploring possible design principles for the achievement of highly selective medicinal agents is by studying compounds that are structurally different from known drugs. Our work is concerned with the design, synthesis, and evaluation of novel medium-sized heterocyclic compounds that can act as ligands of bioamine receptors. In particular, our intention is to explore the effect on affinity in going from rigid, fused-ring compounds such as **1** to medium-ring compounds such as **2**.



1 **2**

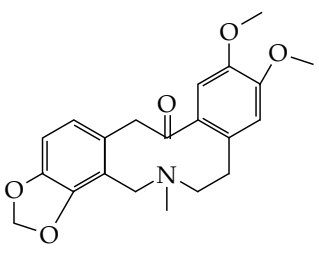
Compound **1** has been synthesized and tested (as the hydrochloride salt, ADT16) and is an α_2 antagonist ($pKi = 8.15$; rat cortex[8]). Compound **2** has a novel chemical structure and offers the potential for the development of a new class of pharmaceuticals. The structural novelty combined with opportunities to modulate the conformational flexibility of medium-ring heterocycles provide a little-explored opportunity[9] to elucidate receptor binding and recognition principles by small molecules. Compound **2** (the ''lead molecule'') also shows interesting conformational features: it is an example of a nine-membered nitrogen-containing heterocycle with torsional constraints. This ''rigid group principle'' to reduce the conformational complexity of medium-sized heterocycles has been employed before,[9] but only a few examples of nine-membered rings have been studied. More importantly, the possibility of a strong intramolecular interaction (the transannular interaction between the nitrogen and the carbonyl carbon) should modify the physical and chemical properties of the lead molecule (**2**).

The transannular interaction of a basic nitrogen with a carbonyl carbon is well established in medium-sized heterocycles.[10–17] Provided the ring is flexible enough, the transannular interaction can lead to a significant shortening of the distance across the ring[14] and even enhance the possibility of transannular cyclization reactions.[11,12] As early as 1922[10] such across the ring electronic effects were first proposed to account for the observed reactivity of the alkaloid cryptopine. The $N \rightarrow C{=}O$ interaction was found by Leonard[11–13] to be more general and it was later used as an experimental basis for mapping the reaction coordinate for the nucleophilic attack of an amine on a carbonyl carbon.[14,17] A range of experimental techniques including NMR, IR, photoelectron spectroscopy, and X-ray crystallography were used to investigate the physical changes in a molecule brought about by the presence of a transannular effect.[11–17] The qualitative nature of the transannular interaction is well understood as a form of through-space homoconjugation,[18] where electron density is donated from the n orbital on the nitrogen into the π^* orbital of the $C{=}O$ bond. However, early attempts at modeling typical compounds with force-field based and semiempirical molecular-orbital based methods[15] and this work demonstrated (see later) that the $N \rightarrow C{=}O$ interaction is not reproduced satisfactorily by these calculations. Our own high level *ab initio* molecular-orbital based calculations [restricted Hartree–Fock (RHF)/631G(d)] were successful in modeling the crystal conformations of compounds which show a transannular interaction between a carbonyl carbon and a nitrogen atom.[19]

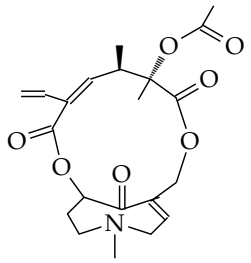
However, it is desirable for the purposes of ligand and structure based computer-aided drug design to be able to accurately describe these molecules using molecular mechanics based methods. This will enable us to use commercially available programs for pharmacophore development and for docking.

The purpose of this article is to present simple modifications to commercially available force fields that achieve this aim. Two test molecules were

chosen from the literature on the basis of structural similarity with the target molecule (**2**) and the availability of X-ray coordinates. Cryptopine (**3**) has a 10-membered heterocycle with similar torsional constraints as the target molecule and clivorine (**4**) has an eight-membered heterocycle with torsional constraints.



cryptopine (**3**)



clivorine (**4**)

Methods

All calculations were performed *in vacuo*. Most calculations were carried out on a Silicon Graphics Iris Indigo workstation using Molecular Simulations Inc. (MSI) molecular modeling software, version 950, principally the InsightII and Discover modules for visualization and computation. The Discover module supports the proprietary CFF91, CVFF, and ESFF force fields as well as the AMBER force field. The new CFF95 force field was also assessed at the MSI offices in Sydney.

The (fractional) X-ray coordinates of the test molecules cryptopine and clivorine were typed in from the original references.[20,21] All molecules modeled were also sketched, converted into 3-dimensional (3-D) structures, and minimized to the closest local minimum using the MSI software.

All input structures were then subjected to molecular dynamics calculations in order to investigate the conformational space available to the molecules. Because the molecules all contain cyclic structures, no systematic searching methods were possible. The protocol was as follows:

1. initialization of dynamics by heating the molecule to 900 K;
2. running N dynamics runs one after the other, each for 1000 $\times$ 1.0 fs (1 ps) at 900 K;
3. the last structure of each dynamics run was sampled and subsequently minimized (steepest descent for 100 iterations, then conjugate gradients) until the maximum derivative was less than 0.0001 kcal/Å or 0.001 kcal/Å for the ESFF force field.

This led to an archive file with N structures that were analyzed further. N was usually 20, but $N = 50$ was also tested in some cases.

Calculations using the Sybyl force field were performed on a Silicon Graphics Indy workstation using Wavefunction Inc.'s Spartan software. The X-ray coordinates mentioned above were used as starting points for performing ''systematic'' conformational searching using the Osawa method,[22] whereby individual ring atoms were alternatively ''puckered up'' and ''puckered down.'' The resulting structures were minimized using the Sybyl force field and duplicates discounted upon geometrical checking. Default settings were used, notably the cutoffs for optimization were as follows: 0.001 Å for the displacements, 0.00001 kcal/mol for the energy, 0.0003 (root mean square, RMS) for the gradients, and 8.0 Å for the van der Waals (vdW) interactions. Selected conformations were exported back into the MSI software for further processing such as superimposition with the crystal structure.

The CFF91 force field was the one selected for modification. The variations used only entailed the introduction of new atom types and parameters associated with these and the analytical expression used by the software for the force field and all other parameters were left untouched.

Molecular dynamics for conformational searching were performed in the edited force fields as described above.

Results and Discussion

UNMODIFIED FORCE FIELDS

Table I summarizes the results obtained for test molecules **3** and **4** using different force fields as compared to the crystal structures obtained by X-ray crystallography.[20,21] From each dynamics or conformational searching run two conformations were picked: the conformation of minimum energy and the conformation of minimum distance across the medium-sized ring between the nitrogen and the carbonyl carbon.

In the crystal structures the carbonyl carbon involved in the transannular interaction was no longer in the plane defined by its three attached atoms. A deviation of 0.213 Å from this plane was

TABLE I.
Results for Two Test Molecules (Cryptopine and Clivorine) after Dynamics in Different Force Fields.

Force Field	Molecule	Energy[a] (kcal / mol)	Distance (Å)
X-ray	Cryptopine	—	2.58
structure	Clivorine	—	1.99
CVFF	Cryptopine	128.1	4.63
		134.4	3.44
	Clivorine	Run failed	++
CFF91	Cryptopine	39.8	3.46
		46.6	2.84
	Clivorine	51.3	3.61
		51.7	2.78
CCF91[b]	Cryptopine	39.4	3.04
		42.8	2.88
	Clivorine	50.8	2.80
		51.9	2.74
CFF95[b]	Clivorine	60.1	2.80
		64.2	2.79
ESFF	Cryptopine	29.6	2.94
		33.3	2.84
	Clivorine	33.2	3.21
		38.2	2.78
AMBER	Cryptopine	50.0	4.59
		52.9	2.90
	Clivorine	Run failed	+++
Sybyl	Cryptopine	22.6	2.70
		27.9	2.63
	Clivorine	21.2	2.70
		26.3	2.57

For each run the minimum energy conformation is shown first and in the second row the conformation of minimum distance (N ⋯ C) across the ring is shown.
[a] Total energy as calculated by the MSI software, except for Sybyl: strain energy as computed by Spartan software.
[b] Out of plane restrictions turned off for the carbonyl carbon. (++) The instability of the Morse bond potential.[25] (+++) Unavailability of parameters for isolated double bonds.

measured for clivorine and 0.102 Å was measured for cryptopine. The restriction for the carbonyl carbon to be in this plane was removed for the runs denoted with the superior letter b in Table I.

This restriction did not, however, allow the two atoms involved in the transannular interaction to approach each other any closer and did not lead to any improvements for either test molecule.

For clivorine it is apparent that the crystal structure cannot be reproduced by any of the force fields tested, except for the Sybyl force field. The Sybyl force field allows the closest distance (2.57 Å), but this conformation bears no resemblance to the crystal structure. A conformation with a distance of 2.61 Å, however, approaches the crystal structure very well, as shown in Figure 1a. The heavy atoms of the medium-sized rings for the two structures have been superimposed in this representation and the resulting RMS deviation noted.

For cryptopine the analogous representation (Fig. 1b) shows excellent agreement between the crystal structure and the conformation found by the Sybyl force field with the smallest distance across the ring. The proprietary MSI force fields cannot reproduce the crystal structure of cryptopine very well at all. The conformations with the smallest distance across the ring determined with the CFF91 and CFF95 force fields are folded, so that the two aromatic rings are stacked. The best approximation within the MSI software was achieved with the AMBER force field and is represented in Figure 1c.

In summary it can be said that none of the proprietary force fields supported in the MSI software can model molecules showing a transannular interaction between a nitrogen and a carbonyl carbon. The Sybyl force field by Tripos, Inc. as supported in the Spartan software, however, can account for a moderate degree of interaction by allowing a closer approach of the two atoms involved. The same seems to be true to a lesser extent for the AMBER force field as supported in the MSI software, but this force field is severely lacking in parameters, for example, for an isolated as opposed to an aromatic sp^2 carbon atom. Neither a recent review[23] nor the AMBER home page on the worldwide web[24] provides references to such parameters either.

COMPARISON OF FORCE FIELDS

The force fields employed in the above study vary widely in their complexity and parameterization. The simplest ones (AMBER, ESFF, and Sybyl) do not use any cross-terms to account for the coupling of various motions like bond stretching and angle bending. The CVFF, CFF91, and CFF95 force fields use a variety of such cross-terms that also necessitates the development of many more parameters. The analytical form and class of compounds used for parameterization for the various force fields are described in the literature.[25–31] The parameters for force fields have usually been derived almost exclusively from experimental data such as crystal structures and thermodynamic properties. The CFF95 force field is an exception to this. Its parameters have been derived mainly from *ab initio* molecular orbital calculations. The Sybyl

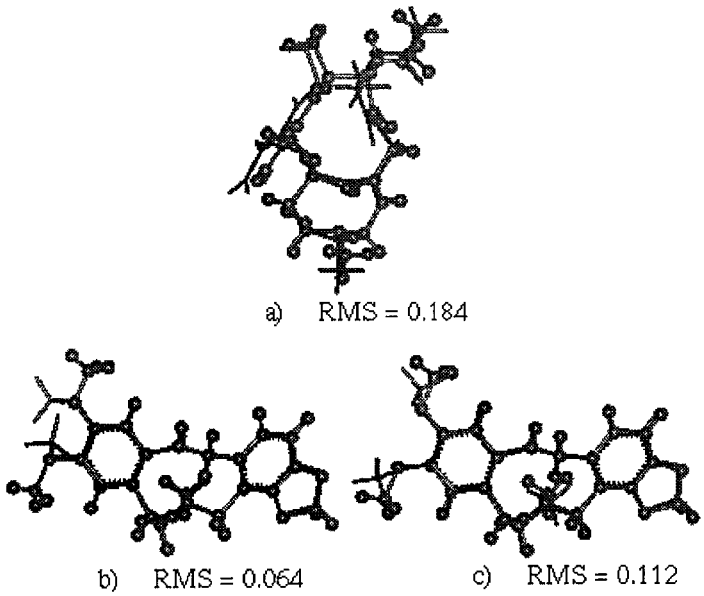
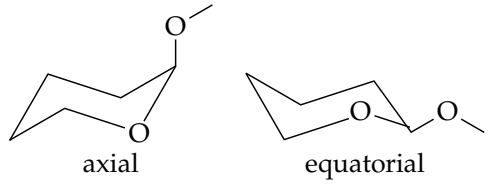


FIGURE 1. Superimpositions of various calculated (heavy lines) conformations with the X-ray structures (ball and stick representation) for (a) clivorine and (b,c) cryptopine. See text for further explanations.

force field stands out in this study as the only one to not use an electrostatic term in its analytical form.

Recently Gundertofte et al.[32] compared a variety of force fields, including CVFF, CFF91, AMBER (as implemented by MacroModel), and Sybyl (as implemented by Alchemy and without the use of charges) in terms of their performance in the calculation of conformational energies for a variety of test molecules. Two of their general conclusions are also borne out in this work. The CVFF force field performs more poorly in all cases than the CFF91 force field and this is also seen here (Table I). The CFF91 force field performs rather poorly in cases where it cannot use explicit parameters. On the other hand, where explicit parameters are at hand, the CFF91 force field performs among the best force fields in the study (MMFF93 and the MM2/MM3 based force fields). It also outperforms AMBER and Sybyl in those cases. To investigate this further, the parameter assignment for clivorine and cryptopine in the CFF91 force field was examined. The assignment of explicit parameters for cryptopine is certainly more complete than for clivorine. For example, the bond parameters for cryptopine are found explicitly for 52 bonds and need to be assigned automatically for one of the bonds. This means that for this one bond, the force field has no parameters defined and parameters from a bond between closely related atom types are substituted. For clivorine, the numbers are 54 and three, respectively. This explains to some extent the findings in Table I, although, as stated above, both the Sybyl and AMBER force fields perform better in this study.

The test molecule 2-methoxy-tetrahydropyran (**5**) in the Gundertofte et al.[32] study is particularly interesting for this work.



conformers of **5**

The axial isomer is favored by 1 kcal/mol due to the anomeric effect. The CVFF and CFF91 force fields both wrongly favor the equatorial isomer whereas AMBER and Sybyl both correctly favor the axial form. The anomeric effect can be explained in terms of two main theories[33]: as a purely electrostatic repulsion or as the delocalization of a lone pair of electrons into an antibonding orbital. The relative importance of the two effects is a matter of controversy and probably depends on the circumstances. What is important in the context of this work is the second or negative hyperconjugation explanation that resembles in principle the explanation of the transannular effect.[18] This helps to rationalize why the transannular effect is

so poorly treated in the CVFF and CFF91 force fields.

Sakakibara and Allinger[34] also recently discussed the failure of molecular mechanics force fields (MM3 in their case) to account for a special interaction that they call ''Lewis bonding.'' They define this type of bonding as the delocalization of electrons from a nucleophilic atom into an antibonding orbital on an electrophilic atom, such as a carbonyl carbon. They define hydrogen bonding as a special case of this Lewis bonding and expect analogously that for any such interaction the two atoms involved will approach closely and that stabilization will result. Obviously, the transannular interaction discussed here also fits their definition.

Another rationale probably lies in the different nonbonded terms. The authors of CFF95[27,28] attribute some of the shortfalls of CVFF to the fact that this force field uses a 12−6 vdW term that is considered to be too hard in its repulsion, and both the CFF91 and CFF95 force fields employ a 9−6 vdW term. Both the Sybyl and AMBER force fields, however, use a 12−6 vdW term, but the difference is in the parameters.

Table II summarizes the nonbonded energies calculated for the interaction between the nitrogen and carbon atoms involved in the transannular interaction. The values were calculated by hand using the parameters belonging to the atom types assigned by the force fields and the analytical expressions used. The distance chosen was 2.58 Å, which is the distance encountered in the crystal form of cryptopine. The cross-over distance between net attraction and repulsion is also shown and, for comparison, the bond-stretching energies for a displacement of 0.1 Å for a carbonyl bond and the total energy of cryptopine are also included. The Sybyl force field certainly shows a much smaller vdW repulsion at the chosen distance, which is additionally much closer to the cross-over distance, regardless of whether this is viewed relative to the total energy of cryptopine or relative to a particular term like the bond-stretching one. The results for the AMBER and CFF91 force fields are of the same order of magnitude.

The treatment of charges also varies between the different force fields. Sybyl does not consider any charges. The charges assigned to the nitrogen and the carbonyl carbon and oxygen involved in the transannular interaction are, however, identical for the CFF91 and AMBER force fields and are as follows: −0.25 for N, +0.40 for C, and −0.40 for O. The partial charges calculated for a model for cryptopine (with the two aromatic rings removed and replaced by double bonds in the 10-membered ring) by optimizing a number of conformations at the RHF/631G(d) level[19] are −0.6 to +0.1 for N, 0.65−0.82 for C, and −0.55 to −0.62 for O. These differ substantially from the ones used by the force fields. In the longer term it is possible that more accurate partial charges can be incorporated into the modified force fields. As can be seen from the small values of the electrostatic term (Table II), this is not expected to result in large changes, however.

MODIFICATION OF CFF91 FORCE FIELD

It is desirable to use force-field based software for drug design and development for reasons of consistency and transferability (whole suites of integrated products are available from different suppliers), ease of manipulation (especially manipulation such as superimposition of families of conformers), and visualization of structures, coupled with the 3-D pattern recognition modules Apex 3D and Catalyst, that are available within the MSI suite of products. For this reason it was decided to modify one of the proprietary MSI force fields to achieve a more accurate description of the model systems. The CFF91 force field was chosen, be-

TABLE II.
Comparison of Selected Energy Terms in Three of Force Fields Used in This Study.

Force Field	van der Waals Energy + Electrostatic Energy (kcal / mol) for d = 2.58 Å	Cross-Over Distance (Attraction = Repulsion) (Å)	Bond-Stretching Energy for Displacement of 0.1 Å for C═O Bond (kcal / mol)	Total Energy of Cryptopine (kcal / mol)
Sybyl	0.80 + 0	2.90	15.6	20 −30[a]
AMBER	8.84 − 0.04	3.70	5.7	~ 50
CFF91	10.98 − 0.04	3.30	6.6	40 −50

[a] Strain energy.

cause it gives the best results before modification. It should be stressed that no attempt was made to derive new parameters in a systematic way, as described in the literature for various force fields.[27,28,35–37] This was considered to be impractical in this case, where only a ''quick fix'' solution was sought to better reproduce experimental geometries.

Two different approaches to the modification of the force field were explored. *The first is that the transannular interaction between a carbonyl carbon and a nitrogen can be treated as a weak bond*. This approach is very similar to the one suggested by Sakakibara and Allinger[34] to account for Lewis bonding in MM3: two parameters need to be estimated, the bond length and the bond strength.

Based on a suggestion in the literature from NMR studies, the strength of this bond was initially set to 5% of that of a normal single bond between a carbonyl carbon and a nitrogen atom.[38] The C═O carbonyl bond strength was accordingly reduced to 95% of its normal value. The bond length for the transannular bond was initially set to 2.581 Å and the carbonyl bond length to 1.209 Å, which are the values found for cryptopine in the crystal. Two new atom types then had to be defined for this bond to be possible: cnew is a carbon atom with parameters identical to the ones for a normal carbonyl carbon (c* in CFF91) except for those specified above. Note that this newly defined carbon is allowed to form five bonds. The newly defined nitrogen atom type is nnew, which is mostly identical to na in CFF91. All torsion parameters involving the cnew-nnew bond were set to zero.

Results from the molecular dynamics treatment of both model molecules with this modified force field, called newCFF91, are summarized in Table III. Only the conformation of minimum energy obtained with each run is listed. Generally it can be said that the conformational flexibility of the medium-sized heterocycles is severely curtailed by the introduction of the additional bond across the ring. For a quick and efficient assessment of this and subsequent force-field modifications, a number of diagnostic distances and angles were measured for each molecule and compared to the values found by X-ray crystallography. These geometrical observables are defined in Figure 2. The presence or absence of a force restraining the carbonyl carbon to coplanarity was not significant in this modified force field, and in all further modifications of this type only the (default) option of the presence of this force will be presented. Interestingly, the results for clivorine were in better overall agreement with the X-ray structure (Table III), even though the X-ray structure for cryptopine was used in the ''parameterization.'' It was felt that the transannular interaction had been overestimated for cryptopine in this modification. We therefore reduced the transannular bond strength to 3% and increased the C═O bond strength to 97%, leaving all other parameters the same. The results with this new version, called new1ACFF91, are also summarized in Table III for cryptopine and no improvement was achieved. Rather than modifying the strength of the transannular interaction further, it was decided to explore the effect of changing the other parameters. In the force field new1BCFF91 the bond strengths were as in newCFF91, but the bond lengths were set to the values for the clivorine crystal form. Table IV summarizes the results for this force field and again it can be seen that no improvement was achieved over newCFF91. In a further modification, called new2CFF91, which was again based on newCFF91, the effect of varying the parameters for torsions was explored. Instead of setting the tor-

TABLE III.
Results for Cryptopine and Clivorine in Modified Force Fields newCFF91 and new1ACFF91.

Molecule	Energy (kcal / mol)	d_1 (Å)	d_2 (Å)	α (degrees)	β_3 (degrees)	β_4 (degrees)	Δ (Å)
Clivorine	74.5	2.22	1.21	120.1	97.5	96.4	0.37
Clivorine	X ray	1.99	1.26	110.2	92.0	93.6	0.21
Cryptopine	61.7	2.37	1.22	118.2	101.5	106.9	0.46
Cryptopine[a]	67.8	2.34	1.22	118.2	101.8	107.3	0.47
Cryptopine	X ray	2.58	1.21	102.2	85.0	95.2	0.10

Values obtained by X-ray crystallography are included for comparison. Distances and angles are defined in Figure 2.
[a] The new1ACFF91.

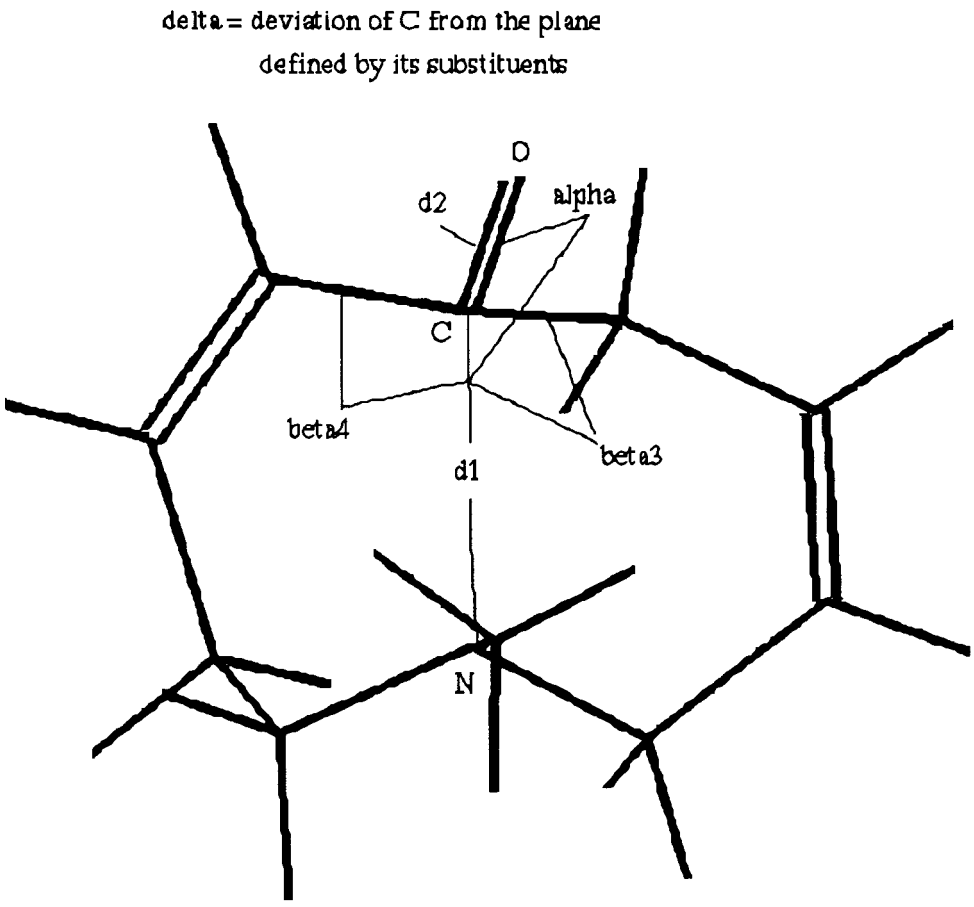


FIGURE 2. Definition of the geometrical observables used in this study.

sion parameters involved in the cnew-nnew bond to zero, they were set to the values employed by CFF91 for amide bonds. Table IV also summarizes the results obtained with new2CFF91. No significant changes can be observed between newCFF91 and new2CFF91. The first modification, newCFF91, was therefore used in further studies (see the following subsection).

Table V further illustrates the performance of the modified force fields for the model compounds. The crystal structures for cryptopine and clivorine were superimposed (as in Fig. 1) onto the calculated structures and very good agreement is observed. The results for clivorine are better than with any of the unmodified force fields.

The second approach to the modification of the CFF91

TABLE IV.
Results for Cryptopine and Clivorine in the Modified Force Fields new1BCFF91 and new2CFF91.

Molecule	Energy (kcal / mol)	d_1 (Å)	d_2 (Å)	α (degrees)	β_3 (degrees)	β_4 (degrees)	Δ (Å)
Clivorine	105.1	1.79	1.27	119.0	98.4	97.9	0.36
Clivorine[a]	151.4	2.19	1.22	119.4	93.0	93.0	0.27
Clivorine	X-ray	1.99	1.26	110.2	92.0	93.6	0.21
Cryptopine	65.9	2.08	1.27	105.5	105.5	110.1	0.52
Cryptopine[a]	93.9	2.38	1.22	118.4	101.4	106.9	0.46
Cryptopine	X-ray	2.58	1.21	102.2	85.0	95.2	0.10

Values obtained by X-ray crystallography are included for comparison. Distances and angles are defined in Figure 2.
[a] The new2CFF91.

TABLE V.
RMS Deviation Values from Superimposition of Selected Conformations of Test Molecules.

Molecule	Force Field	RMS (Å)
Cryptopine	newCFF91	0.140
Clivorine	newCFF91	0.110
Cryptopine	nonCFF91	0.387
Clivorine	nonCFF91	0.114
Target **2**	newCFF91	0.288
Target **2**	nonCFF91	0.631

Values are as calculated with the modified force fields indicated onto the X-ray coordinates or (in the case of **2**) onto ADT16 (**1**) modeled in the unmodified CFF91 force field.

force field involves treating the transannular interaction in a manner similar to the treatment of hydrogen bonds in this force field. This approach was also implicitly suggested by Sakakibara and Allinger.[34] In their definition of Lewis bonding they included hydrogen bonding; therefore, it seemed to be a reasonable approach to treat the transannular interaction in a similar manner.

In the proprietary MSI force fields, ''hydrogen bonds are a natural consequence of the standard van der Waals and electrostatic parameters, and special hydrogen functions do not improve the fit . . . to experimental data.''[25] This means, that a normal hydrogen atom is described by the following set of nonbonded parameters (atom type h):

$$r = 2.9950, \quad \varepsilon = 0.0200,$$

whereas a polar hydrogen bonded to N or O is described by atom type h*:

$$r = 1.0980, \quad \varepsilon = 0.0130.$$

The parameters for the N or O involved in a hydrogen bond remain unchanged.

The square root of the ratio of the parameters for h and h* was used to scale down the nonbond parameters for both rather than just one of the two new atom types nnew and cnew from the normal nonbond parameters for carbonyl carbon (atom type c*) and nitrogen (atom type na). The parameters implemented in this force-field modification, which is called nonCFF91, are given in Table VI. All other parameters for na and c* were used unchanged for nnew and cnew. This shows one large advantage of the ''nonbond'' approach over the ''weak bond'' approach for the treatment of the transannular interaction: only two new parameters need to be specified for each new atom type.

TABLE VI.
Nonbod Parameters Used in nonCFF91 Force Field.

Atom Type	r	ε
na[a]	4.0700	0.0650
c*[a]	3.3080	0.1200
nnew	2.4643	0.0524
cnew	2.0029	0.0967

[a] Taken from CFF91.

In all the nonbond-type force-field modifications at least two different starting conformations were used for the dynamics runs: one conformation with a very short transannular distance and one with a long transannular distance. In Table VII the results are summarized for the two model systems in the nonCFF91 force field. As can be seen for clivorine, and to a lesser extent for cryptopine, this force-field modification does indeed allow the two atoms involved in the transannular interaction to approach closely and can thus model the X-ray structure quite well with very little increase in the total energy. What is also observed is that a number of other low-energy conformations are accessible to the molecule. This was expected and was also observed with our *ab initio* calculations.[19] Only the global minimum structure and the structure with the smallest distance across the ring are shown in Table VII.

The effect of removing the out of plane readjustment force completely for the carbonyl carbon was also investigated (Table VII). This modification does seem to improve the fit of both model systems to the X-ray structure. No other modification was found to improve the performance of the nonCFF91 force field and this was therefore used in further studies (see following subsection).

Table V also illustrates the performance of this modified force field for cryptopine (the cryptopine[a] structure does not bear any resemblance to the crystal structure) and clivorine[a]. The agreement is not very good for cryptopine, possibly due to selection of a ''wrong'' conformation out of the number of possible conformations. For clivorine, there is excellent agreement.

TARGET MOLECULE 2 IN MODIFIED FORCE FIELDS

The target molecule **2** can now be easily modeled in both the modified force fields and compared to compound **1**. Table V also illustrates the

TABLE VII.
Results for Cryptopine and Clivorine in the Modified Force Field nonCFF91.

Molecule	Energy (kcal / mol)	d_1 (Å)	d_2 (Å)	α (degrees)	β_3 (degrees)	β_4 (degrees)	Δ (Å)
Clivorine	47.7	2.45	1.22	95.0	89.0	89.2	0.13
Clivorine[a]	47.7	2.38	1.22	121.3	86.9	89.5	0.26
Clivorine	X-ray	1.99	1.26	110.2	92.0	93.6	0.21
Cryptopine	37.3	3.65	1.23	154.7	46.4	81.1	0.03
Cryptopine	38.0	3.19	1.23	144.2	63.4	75.6	0.04
Cryptopine[a]	36.7	3.68	1.22	153.5	44.5	79.0	0.15
Cryptopine[a]	38.8	2.32	1.22	111.5	105.0	85.3	0.29
Cryptopine	X-ray	2.58	1.21	102.2	85.0	95.2	0.10

Two conformations are shown for each molecule: first the minimum energy conformation and second the conformation of minimum d_1. (For clivorine they coincide.) Values obtained by X-ray crystallography are included for comparison. Distances and angles are defined in Figure 2.
[a] No OOP penalty for the carbonyl carbon.

results of this study. Compound **1** was modeled in the unmodified CFF91 force field and the target molecule was modeled in newCFF91 and nonCFF91. For each run the minimum energy conformation was selected and the two compounds were superimposed using all heavy atoms they have in common. As discussed for cryptopine above, the nonCFF91 force field allows a variety of conformations and it is not immediately obvious which would be the ''best'' one. The newCFF91 force field, however, shows very good agreement. From this study we would therefore predict compound **2** to be able to indeed mimic compound **1** and therefore show affinity for the α_2 adrenergic receptor subtype. Compound **2** is currently being synthesized in our laboratory and its affinity will then be tested *in vitro*.

Conclusions

Our study has shown that different commercially available force fields vary substantially in their treatment of the transannular interaction between a carbonyl carbon and a nitrogen in medium-sized heterocycles. Two model systems with known crystal structures were used to test this. The Sybyl force field by Tripos as implemented in the Spartan package by Wavefunction produced by far the best results and the proprietary CVFF force field as implemented in the MSI software produced by far the worst results of the force fields tested. None of the force fields tested were explicitly parameterized to cope with such an intramolecular interaction, and their variable performance was compared to other force-field comparisons in the literature. An attempt was made to explain this variable performance and it seemed to be mainly correlated to the nonbonded parameters employed by the Sybyl force field, which differ markedly from the ones used in the other force fields.

The proprietary MSI force field CFF91 was then modified in a successful attempt to improve its performance. Two different modifications were studied. In the first type (newCFF91) the transannular interaction was treated as a weak bond. This force field can reproduce the crystal structures of the two model systems very well, but it has the disadvantage of being tedious to implement and, more seriously, of not being able to allow any other (open-ring type) conformations.

The second modification (nonCFF91) treated the transannular interaction similarly to a hydrogen bond by adjusting the nonbonding parameters of the atoms involved. This force field was not quite as successful in reproducing the crystal structures of the model systems but had the twin advantages of being easier to implement and able to reproduce the conformational flexibility shown by the model systems when they were modeled with *ab initio* methods.

Finally, a potentially bioactive compound was studied in the two modified force fields and is predicted, due to the transannular interaction, to be able to mimic the shape of a fused-ring compound that shows biological activity. Thus, it is predicted that this novel medium-sized heterocyclic target compound will show similar α_2 adrenoceptor binding.

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